Comments Regarding Nipples/Areolae Retention Endpoint

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Adverse Effect?

- Are retained nipples in male rodents an adverse effect?
  - may be retained into adulthood, **however**
  - no known effect on function or health
  - not necessarily predictive for other overt adverse anti-androgenic effects (e.g., hypospadias, cleft phallus, ectopic testes) on an individual animal basis (McIntyre *et al.*, 2001);
  - no homologous human issue (human males retain their nipples into adulthood)

Critical Issues

- Do retained areolae/nipples in male rodents signal anti-androgenic potential?
- Are they the most sensitive endpoint or are they redundant to other more overt endpoints that are already characterized?
- Is the method of characterization practical for large scale studies?
- Are the proposed time-points and methods for evaluation feasible, necessary, or redundant?

Signal for anti-androgenic potential?

- Studies that support areolae/nipple retention (A/N R) in male rodents may signal anti-androgenic potential of a compound:
  - Vinclozolin: Gray and Kelce., 1996 and George et al., 2003
  - Procymidone: Gray et al., 1995
  - Linuron: Gray *et al.,* 1999; McIntyre et al, 2000 and 2002
  - Flutamide: McIntyre *et al.*, 2001 and Miyata et al, 2002
  - Fenitrothion: Turner *et al.,* 2002
  - Diethylhexylphthalate (DEHP): Moore *et al.,* 2001
  - Di-n-butylphthalate (DBP): Mylchreest *et al.*, 2000 and George *et al.*, 2003

Comparison of A/N R Sensitivity with Other Endpoints

Compound	Low dose with A/N R↑	Other findings at same or lower dose
Vinclozolin	50 mg/kg	AGD ↓; hypospadias; cleft phallus
Procymidone	100 mg/kg	AGD ↓; hypospadias
Linuron	50 mg/kg	AGD ↓; hypospadias; cleft phallus; ↓ pup survival; ↓ testes wt; **25 mg/kg**: testicular hypoplasia

Comparison of A/N R Sensitivity with Other Endpoints

Compound	Low dose with A/N R↑	Other findings at same or lower dose
Flutamide	6.25 mg/kg	↓AGD; increased cryptorchid/ectopic testes; ↓repro. organ wts
Fenitrothion	25 mg/kg	↓AGD; maternal tox. and ↑fetal death at **20 mg/kg**

Comparison of A/N R Sensitivity with Other Endpoints

Compound	Low dose with A/N R ↑	Other findings at same or lower dose
DEHP	375 mg/kg	Anterior and ventral prostate agenesis; incomplete preputial separation
DBP	100 mg/kg (A only)	Absent Cowpers glands, ↓ testes wt, cranial suspensory ligament (PND 21); cleft phallus, enlarged testes (PND 95)

Sensitivity of A/N R

- In general, other male reproductive toxicity endpoints occurred at equivalent or lower doses to those showing retained A/N at ~ PND 13
- Direct comparison of sensitivity of this endpoint to other parameters assessed in 2-generation reproductive toxicity study is not possible
- Based on current data; no strong evidence that the current 2-generation protocol would miss significant anti-androgenic effects

Interpretation Issues

- Interpretation versus natural background incidence
  - Need for training and criteria for consistency of areolae observations (may be indistinct)
  - Historical control data needed for interpretation—there is a relatively low natural background incidence of retained areolae

A/N R as a Tier Trigger?

- Data from the two phthalate ester studies suggest that an increased count of A/N R ~ PND 13 may provide a signal that detailed evaluation of the male reproductive tract (similar to that done in the one-generation extension study) should be conducted, particularly for adult F1 males
- A/N R ~ PND 13 in males may be a useful tier trigger
- Utility should be evaluated with direct comparisons of sensitivity with current two-generation study required parameters

Practicality Issues

- The practicality of adding multiple endpoints into a large scale study must be carefully assessed
  - Adding count of A/N R ~PND 13
    - ↑ Need for training (especially for consistency of A obs)
    - ↑Time for observation; time may be decreased if done concurrent with bw at PND 14
  - Adding shaving all males at necropsy (PND 21 and 95)
    - Very labor intensive
  - Adding whole mounts of retained nipples and histopathological evaluation
    - Very labor intensive; expensive

Practicality versus Value Added

- In all studies, A/N R ~ PND 13 is a more sensitive endpoint than A/N R at later intervals.
- Evaluation of A/N R into maturity does not provide useful information for risk assessment.
- Position of the retained A/N has not provided critical information, and should not be required.
- No data suggest whole mount evaluation of retained A/N would provide useful information for either hazard characterization or risk assessment. This should not be required.

Recommendations

- There are insufficient data to conclude that addition of A/N R evaluation to a two-generation study would either improve the sensitivity of the assay for hazard assessment or change NOAEL determination for risk assessment; current data suggest this is unlikely.
- Data suggest, however, that A/N R count ~PND 13 may provide a feasible and useful tier trigger to focus additional attention on male reproductive tract evaluation. This strategy should be evaluated for possible future inclusion in the reproductive toxicity study guidelines.